

eta-O Linked to the 1, 2, 3, 4, or 6 position of the adjacent monosaccharide or a linear or branched polysaccharide. H or one of the following: 11 H1

a-O Linked to the 1, 2, 3, 4, or 6 position of the adjacent monosaccharide or a Linear or branched polysaccharide. H or one of the following:

R2 =

H or one of the following: OH, SO<sub>3</sub>, phosphate, NH<sub>2</sub>NHAc, OCH<sub>3</sub>, O-alkyl, or inorganic-alkyl; O linked to another R2-10 within the same monosaccharide. II. R2-10

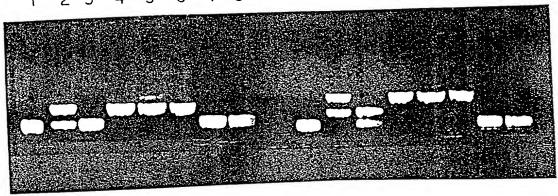
— — — —

113 :  $Gal\beta 1-3G1cNAc\beta 1-3Ga1\beta 1-4G1c-Co$ 

Fuca1-4

167 :  $Gal\beta 1-3G1cNAc\beta 1-3Ga1\beta 1-4G1c-Co$ 

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



# Substrate 113

Oub.	J C. J	
1	+	no preparation
2	+	X. holcicola preparation
3	+	X. badrii preparation

4 + X. manihotis preparation
5 + X. cyanopsidis preparation

6 + X. oryzae preparation

7 + X. campestris preparation

8 + X. campestris preparation

# Substrate 157

Cubb		
9	+	no preparation
10	+	X. holcicola preparation
11	+	X. badrii preparation
12	+	X. manihotis preparation
13	+	X. cyanopsidis preparation
14	+	X. oryzae preparation
15	+	X. campestris preparation
16	+	X. campestris preparation

109 : Ga1α1-3Ga1β1-3G1cNAc-Co

# M | 2345678910

```
Substrate 109
                            complete digest
Lanes 1-4
                            1 \mul. of \alpha1-3, 6 Galactosidase
      1
                     =
                            0.5 \mu l.
      2
                            0.25~\mu l. : concentration of enzyme-4 units/\mu l.
      3
                            0.125 \mu l.
      4
                     partial digest
      5-8
                     undigested
      9-10
```

FIG.3

120 : Fucα1-2Galβ1-4G1c-Co

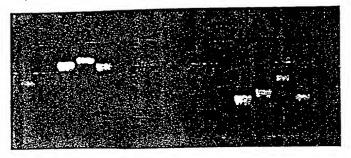
95 :  $Ga1\beta1-4G1cNAc\beta1-3Ga1\beta1-4G1c-Co$ 

→/ Fuca1-3

113 :  $Ga1\beta1-4G1cNAc\beta1-3Ga1\beta1-4-Co$ 

→ / Fuca1-4

# M 123 4567 891011



### Substrate 120

1 + no enzyme

2 + α-Fucosidase II 3 + α-Fucosidase I

### Substrate 95

4 no enzyme

5 + α-Fucosidase I

6 +  $\alpha$ -Fucosidase I +  $\beta$ -Galactosidase (bovine testes)

7 + α-Fucosidase II

### Substrate 113

8 no enzyme

9 + a-Fucosidase I

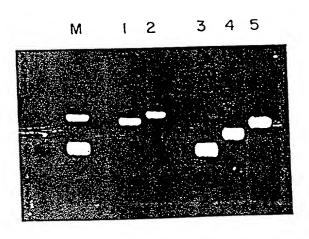
10 +  $\alpha$ -Fucosidase | +  $\beta$ -Galactosidase (bovine testes)

11 + a-Fucosidase II

FIG. 4

118 : Ga1CnaC $\beta$ 1-4G1cNAc $\beta$ 1-4G1cNAc-Co

167 :  $Ga1\beta1-3G1cNAc\beta1-3Ga1\beta1-4G1c-Co$ 



# Substrate 118

1 + no enzyme 2 +  $\beta$ -GlcNAcase

# Substrate 167

3 + no.enzyme

4 +  $\beta$ -Galactosidase

5 +  $\beta$ -Galactosidase +  $\beta$ -G1cNAcase

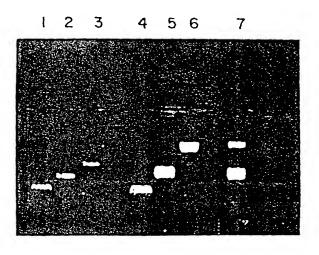
FIG.5

200 :  $Ga1\beta1-4G1cNAc\beta1-2Man\alpha1-6Man\beta1-4G1c-Co$ 

(linear)

197 :  $Ga1\beta1-4G1cNAc\beta1 \setminus 6 Ga1\beta1-4G1c-Co$ 

(branched)  $Ga1\beta1-4G1cNAc\beta1/3$ 



## Substrate 200

1 + no enzyme

2 +  $\beta$ -Galactosidase

3 +  $\beta$ -Galactosidase +  $\beta$ -GlcNAcase (X.manihotis)

# Substrate 197

4 + no enzyme

5 +  $\beta$ -Galactosidase

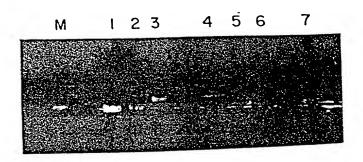
6 +  $\beta$ -Galactosidase +  $\beta$ -G1cNAcase (X. manihotis)

7 + Marker (92b, 167)

FIG.6

. Ga1NAcβ1-3Ga1α1-4Ga1β1-4G1c-Co 96

 $Ga1NAc\beta1$ -4 $Ga1\beta1$ -4G1c-Co 205



# Substrate 96

+ no enzyme

 $\beta$ -GlcNAcase (X. manihotis) 2 β-GlcNAcase) (bovine kidney) 3

# Substrate 205

no enzyme 4

 $\beta$ -GlcNAcase (X. manihotis) 5

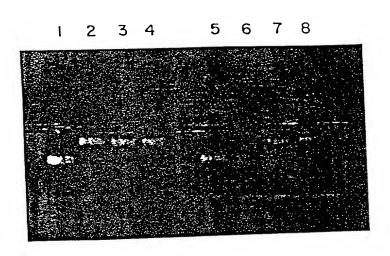
β-GlcNAcase (bovine kidney) 6 +

Marker (92b, 167) 7

FIG.7

 $Ga1\beta1-3G1cNAc\beta1-3Ga1\beta1-4G1c-Co$ (167):

 $Ga1\beta1-4G1cNAc\beta1-3Ga1\beta1-4G1c-Co$ (202):



### Substrate 167

no enzyme

 $\beta$ 1-3>>4 Galactosidase (X. manihotis) at 1x concentration 2

 $\beta$ 1-3, 4>6 Galactosidase (bovine testes) at 1x concentration 3

 $\beta$ 1-3, 4 Galactosidase (chicken liver) at 1x concentration

# Substrate 202

no enzyme

 $\beta$ 1-3>>4 Galactosidase (X. manihotis) at 100x concentration 6

 $\beta$ 1-3, 4>6 Galactosidase (bovine testes) at 1x concentration 7

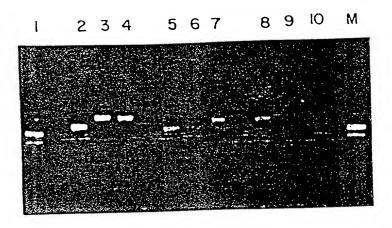
 $\beta$ 1-3, 4 Galactosidase (chicken liver) at 1x concentration 8

FIG. 8

Ga1α1-3Ga1β1-3G1cNAc-Co 109

 $Ga1\alpha 1-4Ga1\beta 1-4Gal-Co$ 193

Ga1a1-6G1ca1-2Fru-Co 181



### Marker 1

## Substrate 109

no enzyme 2

a1-3, 6 Galactosidase (X. manihotis) 3

a1-3, 4, 6 Galactosidase (coffee bean)

### Substrate 193

no enzyme 5

a1-3, 6 Galactosidase (X. manihotis) 6

a1-3, 4, 6 Galactosidase (coffee bean) 7

## Substrate 181

no enzyme 8

a1-3, 6 Galactosidase (X. manihotis) 9

a1-3, 4, 6 Galactosidase (coffee bean) 10

134 :  $Man\alpha 1 - 2Man\alpha 1 - 3Man\beta 1 - 4G1cNAc-Co$ 

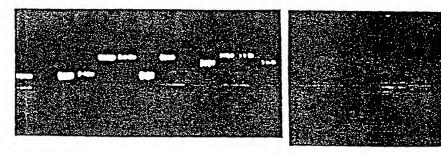
1, 111

114 :  $\operatorname{Man} \alpha 1^{\frac{1}{3}} \operatorname{Man} \beta 1-4 \operatorname{G1cNAc-Co}$ 

200 :  $Gal\beta 1-4G1cNAc\beta 1-2Man\alpha 1-6Man\beta 1-4GlcNAc-Co$ 

1, 11

### M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



### Substrate 134

- 1 + no enzyme
- 2 + a-Mannosidase I (15 units, 20 hrs.)
- 3 + α-Mannosidase III (15 units, 2 hrs.)
- 4 +  $\alpha$ -Mannosidase III (15 units, 20 hrs.)
- 5 +  $\alpha$ -Mannosidase II (100 units, 20 hrs.)
- 6 + Jack bean α-Mannosidase

### Substrate 114

- 7 + no enzyme
- 8 +  $\alpha$ -Mannosidase I (15 units, 2 hrs.)
- 9 + a-Mannosidase III (15 units, 2 hrs.)
- 10 +  $\alpha$ -Mannosidase III (15 units, 2 hrs.)
- 11 + α-Mannosidase II (100 units, 20 hrs.)

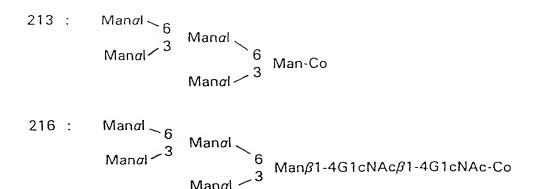
### Substrate 200

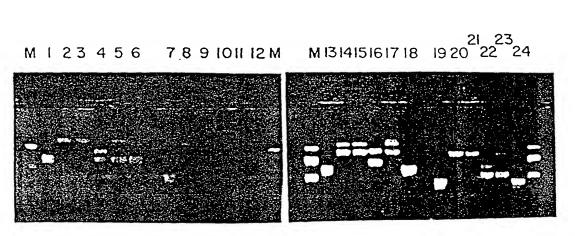
- 12 + no enzyme
- 13 +  $\beta$ -Galactosidase (bovine testes<sup>OGS)</sup>
- 14 +  $\beta$ -Galactosidase +  $\beta$ -GlcNAcase

units, 2 hrs.)

- 15 +  $\beta$ -Galactosidase +  $\beta$ -GlcNAcase +  $\alpha$ -Mannosidase I (15
- units, 2 hrs.)

  16 +  $\beta$ -Galactosidase +  $\beta$ -GlcNAcase +  $\alpha$ -Mannosidase III (15)
- 17 +  $\beta$ -Galactosidase +  $\beta$ -GlcNAcase +  $\alpha$ -Mannosidase II (15 units, 2 hrs.)





### Substrate 213 Substrate 213 13 + no enzyme 1 + no enzyme 2 + a-Mannosidase I 14 + a-Mannosidase I 3 + q-Mannosidase I + II 15 + a-Mannosidase 1 + 114 + a-Mannosidase III 16 + a-Mannosidase III 5 + a-Mannosidase II + III 17 + α-Mannosidase II + III 6 + a-Mannosidase II 18 + a-Mannosidase II Substrate 216 Substrate 216 7 + no enzyme 19 + no enzyme 8 + a-Mannosidase I 20 + α-Mannosidase I $9 + \alpha$ -Mannosidase 1 + 1121 + $\alpha$ -Mannosidase I + II 10 + α-Mannosidase III 22 + a-Mannosidase III 11 + a-Mannosidase II + III 23 + $\alpha$ -Mannosidase II + III 12 + α-Mannosidase II 24 + a-Mannosidase II

TWENTY-HOUR INCUBATION

TWO-HOUR INCUBATION

179 : Glcβ1-4Glcβ1-4Glc-Co

180 : Glca1-4Glca1-4Glc-Co

118 : GlcNAcβ1-4GlcNAcβ1-4GlcNAc-Co

202 :  $Gal\beta 1-4GlcNAc\beta 1-3Gal\beta 1-4Glc-Co$ 



### M Marker

### Substrate 179

1 + no enzyme

 $2 + \beta$ Glucosidase (1 unit)

### Substrate 180

3 + no enzyme

 $4 + \beta$ Glucosidase (5 units)

### Substrate 118

5 + no enzyme

 $6 + \beta$ Glucosidase (5 unit)

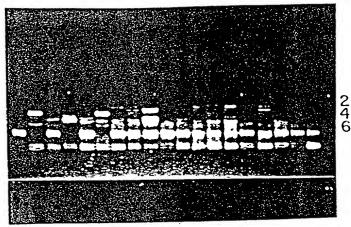
 $7 + \beta$ blcNAcase

### Substrate 202

8 + no enzyme

9 +  $\beta$ Glucosidase (5 units)

10 +  $\beta$ Galactosidase



1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 17 18

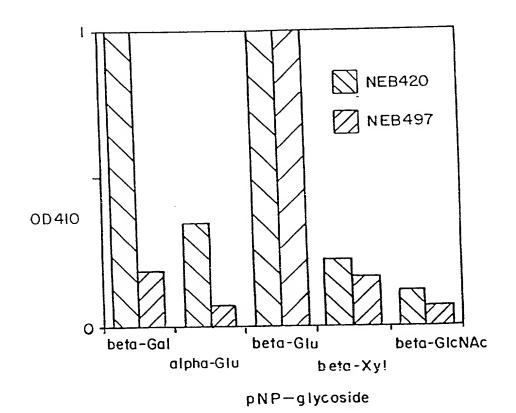
Substrate: Gs 300

## Lane Nos.

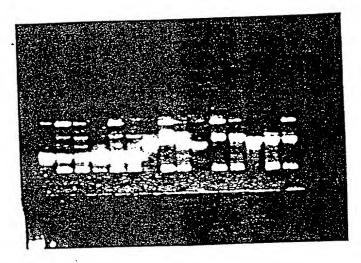
١.	No extract
2.	Xanthomonas holicicola ATCC # 13461
3.	Xanthomonas badrii ATCC # 11672
4.	Xanthomonas manihotis ATCC # 49764
5.	Xanthomonas cyanopsidis ATCC # 55472
6.	Xanthomonas oryzae ATCC # 55470
7.	Xanthomonas campestris ATCC # 55470
8.	Xanthomonas campestris

M: Markers (92b, 167, 197)

9.	No extract	
10.	Bacillus globigii l	
11.	Bacillus globigii II	
12.	Bacillus caldolyticus	
13.	Bacillus brevis	
14.	Bacillus stearothemorphilus	Strain A
15.	Bacillus stearothemorphilus	Strain B
16.	Bacillus aneurinolyticus	
17.	Bacillus sphaericus	_
18.	Bacillus stearothermophilus	Strain C

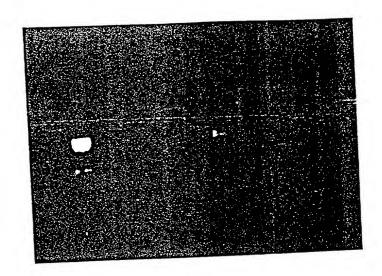


F1G.14



1 23 4 5 6 7 8 9 10 11 12 13 14 15

### Substrate 202 1. No extract **NEB 420** 2. Xanthomonas campestris **NEB 497** 3. Xanthomonas campestris Substrate 167 4. No extract **NEB 420** 5. Xanthomonas campestris **NEB 497** 6. Xanthomonas campestris Substrate 180 7. No extract **NEB 420** 8. Xanthomonas campestris **NEB 497** 9. Xanthomonas campestris Substrate 179 10. No extract **NEB 420** 11. Xanthomonas campestris 12. Xanthomonas campestris **NEB 497** Substrate 233 13. No extract **NEB 420** 14. Xanthomonas campestris **NEB 497** 15. Xanthomonas campestris



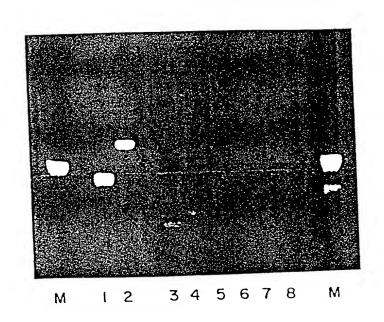
M: Marker (191, 202)

# Substrate 300

- 1. No enzyme
- 2. 2 units σ1-2, 3 Mannosidase (Xanthomonas manihotis)
- 3. 2 units  $\sigma$ 1-2. 3 Mannosidase + 5 units  $\beta$ -Xylosidase (Xanthomonas holcicola)
- 4. 5 units  $\beta$ -Xylosidase (Xanthomonas holicola)

# Substrate 264

- 1. No enzyme
- 2. 5 units  $\beta$ -Xylosidase (Xanthomonas holicicola)



M: Marker (191, 202)

# Substrate 259

- No enzyme
- 2. 2.5 units  $\beta$ -Mannosidase

# Substrate 300

- 3. No enzyme
- 4. 2 units a1-2, 3 Mannosidase (Xanthomonas manihotis)
- 5. 2 units  $\alpha$ 1-2, 3 Mannosidase + 2 units  $\beta$ -Xylosidase (Xanthomonas holcicola)
- 2 units α1-2, 3 Mannosidase + 2 units β-Xylosidase + 10 units α1-6
   Mannosidase (Xanthomonas manihotis)
- 7. 2 units  $\alpha$ 1-2, 3 Mannosidase + 2 units  $\beta$ -Xylosidase + 10 units  $\alpha$ 1-6 Mannosidase + 2.5 units  $\beta$ -Mannosidase (Xanthomonas holcicola)
- 8. 2.5 units  $\beta$ -Mannosidase (Xanthomonas holcicola)